Influence of spermine on intestinal maturation of the glycoprotein glycosylation process in neonatal rats

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Previous work has shown an inverse evolution of the rat intestinal glycoprotein sialylation that decreases from birth to weaning and of fucosylation that increases markedly after weaning during postnatal development. At weaning time, an increase in the intestinal level of polyamines (and especially that of spermine) was observed, owing partly to the higher level of spermine found in solid food given to rats at this period in comparison with the level found in milk. To study the role of this polyamine as a possible maturation factor of the glycoprotein glycosylation, suckling rats were treated for 4 days with spermine administered orally. This treatment allowed us to mimic the spermine increase that was observed naturally in rat small intestine after weaning because, in intestines of spermine-treated suckling rats, spermine was the only polyamine to be increased and was at a level similar to that of weaned rats. Spermine treatment did not induce appreciable changes in sialyltransferase activity or in sialylation of the brush-border-membrane glycoproteins. On the contrary, this treatment induced a rise in an α-1,2-fucosyltransferase activity that was regulated at the transcriptional level, but not by its inhibitor (fuctinin), and no change in the availability of substrate (GDP-fucose). As a consequence of the increase in α-1,2-fucosyltransferase level and of the decrease in α-L-fucosidase level after treatment with spermine, several α-1,2-fucoproteins, naturally found in brush border membranes after weaning time, appeared precociously in these membranes after the treatment of the immature suckling rats. These results indicate that spermine is a maturation factor for the fucosylation of intestinal brush-border-membrane glycoproteins but not for their sialylation, and that this polyamine might be implicated in the increased fucosylation naturally occurring at weaning time during postnatal development.

Key words: development, fucosyltransferase, intestine, polyamine, sialyltransferase.

INTRODUCTION

During postnatal development, the mucosa of rat small intestine undergoes changes affecting morphological and functional characteristics. At the end of the third postnatal week, intestinal maturation is associated with increased mucosal proliferation and changes in the activity of the digestive enzymes (e.g. lactase, sucrase, maltase and aminopeptidase) that enable the animal to cope with the solid diet fed at weaning [1]. Most of these enzymes are glycoproteins but the role of the glycan chains of these glycoproteins on their activity is not clearly understood. Developmental variations in the glycosylation of intestinal glycoproteins have also been observed. A shift from a high sialylation before weaning to a high fucosylation of the glycan chains after weaning has been demonstrated in glycoproteins of purified brush border membranes [2–4] and in mucins [5] by various techniques, as well as in the apical and basolateral membranes of the epithelial cells by lectin cytochemistry [6]. This shift is accompanied by a parallel decline in the activity and the mRNA level of an α-2,6-sialyltransferase from birth to weaning time [7–9] and by a large increase in the activity of an α-1,2-fucosyltransferase just after weaning [10,11].

The factors regulating the developmental changes in intestinal morphology, enzymic activities and in glycoprotein glycosylation during the postnatal period are still incompletely understood. Postnatal digestive tract maturation is controlled by hormonal factors such as corticoids, thyroxine or insulin [1,7,11–14] but other growth or maturation factors such as polyamines are also strong candidates for the regulation of the intestinal maturation. In mammals, a continuous supply of polyamines is vital for the renewal and functioning of the gut epithelium and the role of polyamines in growth and differentiation is particularly important. In rat small intestine, increased ornithine decarboxylase activity and polyamine levels occur during the weaning period [15] in association with the other mucosal modifications. The intake of dietary polyamines, added to their biosynthesis, is important in this process [16,17] and the importance of polyamines from extracellular sources, particularly from the diet, is increasingly being emphasized [18]. The oral administration of polyamines to suckling rats caused the precocious disappearance of lactase and the appearance of sucrase and maltase activities [19–22]. Although polyamines are known to induce precocious variations in the activity of some glycoprotein digestive enzymes in suckling rats, the implication for polyamines in the regulation of the intestinal glycoprotein glycosylation has never been studied.

Here we examine the effect of spermine ingestion by suckling rats on the sialylation and fucosylation of glycoproteins in small intestine. The aim of this study was to find out whether polyamines might have a role in the regulation of the sialylation and fucosylation of intestinal glycoproteins during postnatal development.

MATERIALS AND METHODS

Animals and treatment

At 1 day after birth, pups were distributed as litters of 10 suckling male rats (Sprague–Dawley strain; IFFA CREDO, L'Arbresle, France), which were maintained at controlled temperature

Abbreviation used: G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

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(21 °C) on a 12 h light–12 h dark cycle. Dams were fed with a solid commercial diet (Ico; UAR, Villemoisson sur Orge, France) and male rats (28 or 48 days of age) were weaned on the same diet when they were 19 days old.

In each litter of 10-day-old suckling rats, a first group was given 0.4 μmol of spermine/g of body weight in water, administered orally, once a day for 4 days starting on the 10th day of life (Spm group). In the same litter, a second group received water alone (Control group). To avoid stress, spermine was not given by gastric cannula but directly into the mouth by means of a syringe with a soft flexible tip. Pups were killed by decapitation at 14 days of age.

Collection of milk

Rat milk was obtained from anaesthetized rats injected with 5 m-i.u. of oxytocin/g body weight to stimulate milk ejection. Milk samples (250–700 μl) were collected the 14th day of lactation by manual expression, put in cryotubes and immediately frozen at −180 °C until polyamine assay.

Cell fractionation

At the end of the treatment, the animals were killed; the small intestines were removed after laparotomy and then flushed with ice-cold 0.9 % NaCl.

For the preparation of microsomes and cytosols, the mucosae were harvested with a glass slide and homogenized in 10 mM Tris/HCl/10 mM KCl/10 mM $MgCl_2$/250 mM sucrose (pH 7.4) with a Potter–Elvehjem homogenizer (5 ml/g of wet tissue). The homogenate was centrifuged at 30000 ***g*** for 30 min and the supernatant was centrifuged at 150000 ***g*** for 90 min at 4 °C, yielding microsomal pellets and cytosols.

The brush border membranes were prepared by the $CaCl_2$ precipitation technique of Kessler et al. [23].

Determination of glycosyltransferase activities

Sialyltransferase activity was determined in the microsomal fraction by using asialofetuin (an acceptor able to link sialic acid in the α-2,3 or α-2,6 positions on the galactose termini of N- or O-glycans) as the exogenous acceptor. The reaction mixture (incubated for 30 min at 30 °C) contained between 150 and 250 μg of proteins, 20 μM asialofetuin, 10 mM AMP, 0.5 % (v/v) Triton X-100, 5 μM CMP-[^{14}C]neuraminic acid (specific radioactivity 10.1 GBq/mmol; Dupont–NEN, Boston, MA, U.S.A.).

Glycoprotein:fucosyltransferase activity was measured with asialofetuin as an exogenous acceptor in the cytosol or the microsomal fraction. Asialofetuin is an acceptor that allows the determination of the α-1,2 linkage of fucose to the galactose residue and the α-1,3 linkage of fucose to the *N*-acetylglucosamine residue of the galactose-(β1,4)-*N*-acetylglucosamine termini of the oligosaccharide chains. The reactions were performed for 30 min at 23 °C in a mixture containing 150–250 μg of proteins, 20 μM asialofetuin, 5 mM $MnCl_2$, 10 mM AMP, 0.25 % (v/v) Triton X-100 and 6.5 μM GDP-[^{14}C]fucose (specific radioactivity 10.0 GBq/mmol; Dupont–NEN). The α-1,2-fucosyltransferase activity (galactoside 2-α-L-fucosyltransferase, EC 2.4.1.69) was determined more specifically with 50 mM phenyl-β-galactopyranoside as the acceptor instead of asialofetuin. The cytosolic fucosyltransferase activities were determined without Triton X-100.

When the exogenous acceptor was a glycoprotein, the reactions were stopped with 20 % (w/v) trichloroacetic acid, the radioactive products were collected on GF/B glass fibre filters (Whatman, Maidstone, Kent, U.K.) and the radioactivity was measured with toluene scintillator (Packard, Downers Grove, IL, U.S.A.). When the exogenous acceptor was phenyl-β-D-galactoside, the reactions were stopped with 3 vol. of ice-cold water; the fucosylated phenyl-β-D-galactoside was separated from GDP-fucose by chromatography on a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) by the method of Palcic et al. [24] and radioactivity was determined after the evaporation of solvents. The results were expressed after subtraction of the endogenous activities (determined without exogenous acceptor).

Other enzymic and regulatory activities involved in the fucosylation process

The synthesis and degradation of the GDP-fucose substrate were studied in the cytosol and their reaction products were separated by HPLC as described previously [25]. α-L-Fucosidase activity was determined by the method of Kobata [26].

The activity of fuctinin (a fucosyltransferase inhibitor) was determined as described previously [25], in a fraction semi-purified from cytosol with partly purified α-1,2-fucosyltransferase [27]. The determination of this activity was based on linear fits obtained from curves of fucosyltransferase activity in the presence of different concentrations of the inhibitor. One activity unit was defined as the quantity of protein giving 50 % inhibition of the fucosyltransferase activity in a standardized assay as described previously [27].

cRNA probes

Two fragments of cDNA encoding for two α-1,2-fucosyltransferases, FTA and FTB [28], were kindly donated by Dr. Jean Philippe Piau (INSERM U419, University of Nantes, Nantes, France) as an insert in a plasmid including a T7 promoter. Digoxigenin-labelled cRNA probes were synthesized from the fragments by using a commercial digoxigenin RNA labelling kit (SP6/T7) from Boehringer Mannheim (Germany). A digoxigenin-labelled cRNA probe for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was prepared from a G3PDH human cDNA fragment linked to a T7 promoter (Clontech, Palo Alto, CA, U.S.A.).

RNA isolation and Northern hybridization analysis

Total RNA, extracted from the mucosa of small intestine (scraped off with a glass slide under sterile conditions) by the guanidinium thiocyanate method of Chomczynski and Sacchi [29]. RNA (25 μg for each sample per lane), was separated by electrophoresis on a 1.2 % (w/v) agarose/2.2 M formaldehyde gel, in Mops buffer for 2 h at 70 V, then blotted by capillarity for 16 h to a nylon membrane (Hybond N; Amersham, Braunschweig, Germany) in 20 × SSC (SSC is 0.15 M NaCl/0.015 M sodium citrate). After prehybridization for 2 h at 68 °C in 5 × SSC/50 % (v/v) formamide/0.2 % SDS/0.1 % *N*-lauroylsarcosine/5 % blocking reagent (Boehringer Mannheim), the membranes were hybridized at 68 °C for 16 h to one of the digoxigenin-labelled cRNA probes for α-1,2-fucosyltransferase FTA or FTB (80 ng/ml) in the same buffer. The membranes were washed at 68 °C successively with 2 × SSC/0.1 % SDS for 10 min, 0.5 × SSC/0.1 % SDS for 15 min and then twice with 0.1 × SSC/0.1 % SDS for 15 min. After washing, chemiluminescence signals were detected on Biomax ML film (Kodak, Rochester, NY, U.S.A.) with a digoxigenin-luminescence kit (Boehringer Mannheim) and quantified and analysed with a Bio-Rad GS-700 densitometer and Molecular Analyst software (Bio-Rad, Hercules, CA, U.S.A.). After stripping of the membranes, control hybridization was conducted in

the same way with the digoxigenin-labelled G3PDH cRNA probe (50 ng/ml).

Detection of sialoproteins and fucoproteins

Proteins of the intestinal brush border membranes were resolved by electrophoresis on a 0.1 % SDS/7.5 % (w/v) polyacrylamide gel, then electrotransferred to a nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). The α-2,3- and α-2,6-sialoproteins were detected by lectin sorbent assays performed in 50 mM Tris/150 mM NaCl/0.1 % (v/v) Tween 20, with digoxigenin-labelled lectins (5 μg/ml) of *Maackia amurensis* (MAA) and of *Sambucus nigra* (1 μg/ml) (SNA) (Boehringer Mannheim) respectively. Digoxigenin was then recognized with an alkaline-phosphatase-labelled anti-digoxigenin antibody (750 m-i.u./ml) (Boehringer Mannheim), followed by the detection of alkaline phosphatase with 1.8 mM Nitro Blue Tetrazolium/0.5 mM 5-bromo-4-chloroindol-3-yl phosphate. The α-1,2-fucoproteins were detected by using a biotin-labelled lectin (0.6 μg/ml) of *Ulex europeus* (UEA-I) (Sigma, St. Louis, MO, U.S.A.) and alkaline-phosphatase-labelled streptavidin (1 i.u./ml) (Boehringer Mannheim), as described previously [25].

Polyamine determination

Polyamine contents were determined by HPLC with the use of a modified procedure of the technique of Wickström and Betner [30]. Samples (milk, commercial food or intestinal mucosa homogenized in deionized water) were diluted and added to 4 μM of 1,6-hexanediamine as internal standard. The samples were precipitated with 1 vol. of 10 % (w/v) trichloroacetic acid for 15 min at 4 °C to eliminate proteins and to permit the efficient extraction of polyamines. They were then centrifuged at 4000 ***g*** for 10 min; the clear supernatants were carefully removed and neutralized at pH 7.0 with 1.2 vol. of 1 M sodium borate, pH 8.5, before the quantification of polyamines. The derivatization of polyamines was performed with 1 mM fluoren-9-ylmethyl chloroformate for 45 s at room temperature and was achieved by the addition of 5 mM glycine. The quantification of polyamines was performed by HPLC after separation on a reverse-phase C_{18} column (150 mm × 4.6 mm, 5 μm particle diameter; Ultrasphere-ODS; Beckman) with a precolumn packed with the same support (45 mm × 4.6 mm) on a 625LC HPLC system (Waters). Fluorescence was monitored with a Waters 474 scanning fluorescence detector with excitation at 260 nm and emission at 315 nm. Polyamines were eluted as follows: the first step was an isocratic phase with 75 % (v/v) solvent A [70 % (v/v) 50 mM acetic acid (pH 4.2)/30 % (v/v) acetonitrile] and 25 % (v/v) solvent B (acetonitrile) for 3.5 min, followed by a non-linear gradient to 38 % (v/v) solvent A/62 % (v/v) solvent B for 21.5 min, then a linear gradient to 100 % solvent B for 2.5 min; the second step was an isocratic phase in the same solvent for 5 min, at a flow rate of 1.5 ml/min. A return to the initial step was obtained by a linear gradient for 2.5 min. Polyamines were identified by retention times and routinely compared with standards. They were quantified by comparison with concentration curves determined for each standard polyamine, with the use of an internal standard.

Chemical determinations

The DNA contents of the homogenates prepared from intestinal mucosae were determined by the method of Burton [31] and their protein contents by the technique of Lowry [32]. Proteins in subcellular fractions were determined by the method of Schaffner and Weissmann [33].

Statistical analysis

The results are expressed as means ± S.E.M. and were compared by the Mann–Whitney *U* test or by Student's *t* test. Results were considered significantly different when the *P* values were < 0.05.

RESULTS

Spermine content in diet and intestine of suckling and weaned rats

The commercial diet (I_{CO}) given to rats at weaning time contained more polyamines and especially more spermine (16.1 ± 2.5 nmol of spermine per g of food; $n = 11$) than milk (2.7 ± 0.7 nmol of spermine per ml of milk on the 14th day of lactation; $n = 16$, $P < 0.001$).

The spermine level in small-intestinal mucosa was higher after weaning (785 ± 91 nmol/g of mucosa, $n = 12$) in 28-day-old rats than in 14-day-old suckling rats (348 ± 69 nmol/g of mucosa; $n = 10$, $P < 0.005$).

Effect of spermine treatment of suckling rats on the intestinal polyamine content

The increase in polyamine level observed after weaning is accompanied by an increase in glycoprotein fucosylation [25,27]. To determine the role of spermine in the intestinal maturation of the glycoprotein fucosylation in small intestine, we tried to obtain an increase in the spermine intestinal content of suckling rats to a level near that of weaned rats, by the oral administration of spermine.

After treatment of suckling rats with spermine, the polyamine content was determined in small-intestinal mucosa (Figure 1). The spermine level in spermine-treated rats (836 ± 138 nmol/g of mucosa; $n = 10$) was increased significantly by a factor of 2.4 compared with that of control rats (348 ± 69 nmol/g of mucosa; $n = 10$, $P < 0.010$), reaching a level similar to that of the weaned 28-day-old rats (785 ± 91 nmol/g of mucosa; $n = 12$, $P > 0.05$, ie. not significantly different from spermine-treated rats). Therefore the percentage of spermine was increased, because it was 47.2 ± 2.0 % ($n = 10$) of total polyamines for the spermine-

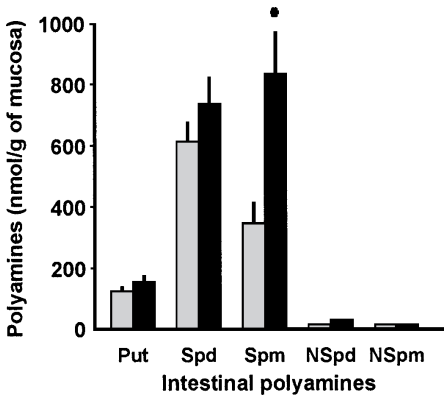


Figure 1 Effect of treatment of suckling rats with spermine on intestinal polyamines

Animals were given 0.4 μmol of spermine/g of body weight orally once a day for 4 days starting on the 10th day of life (spermine group, black bars), or water in the same way (control group, grey bars), then killed on the 14th day of life. Polyamine content was determined by HPLC. Abbreviations: Put, putrescine; Spd, spermidine; Spm, spermine; NSpd, *N*-acetylspermidine; NSpm, *N*-acetylspermine. Results are given as means ± S.E.M. ($n = 10$) and were compared by the Mann–Whitney *U* test. $^*P < 0.005$.

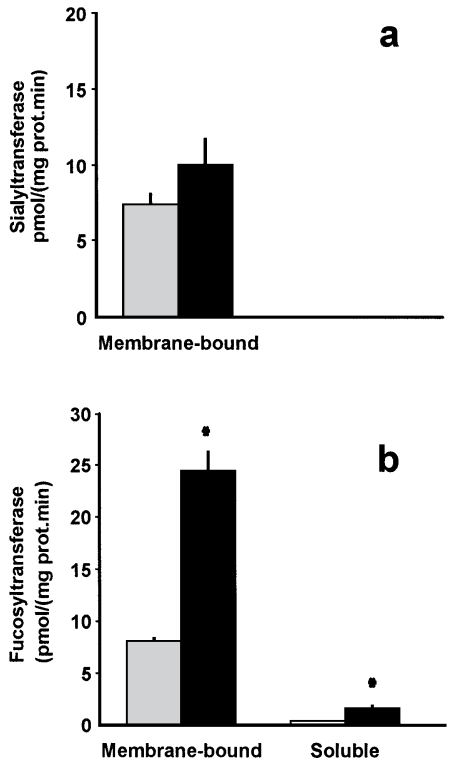


Figure 2 Effect of treatment of suckling rats with spermine on sialyltransferase and fucosyltransferase activities

Animals were given 0.4 μmole of spermine/g body weight orally once a day for 4 days starting on the 10th day of life (spermine group, black bars), or water in the same way (control group, grey bars), then killed on the 14th day of life. (**a**) Sialyltransferase activity, determined in the microsomal fraction with asialofetuin as exogenous acceptor. Means $\pm$ S.E.M. (12 values) were compared by the Mann–Whitney *U* test. (**b**) Fucosyltransferase activity determined in the microsomal fraction (membrane-bound) and in the cytosol (soluble) with asialofetuin as exogenous acceptor. Means $\pm$ S.E.M. (15 values) were compared by the Mann–Whitney U test. $^{*}P < 0.001$.

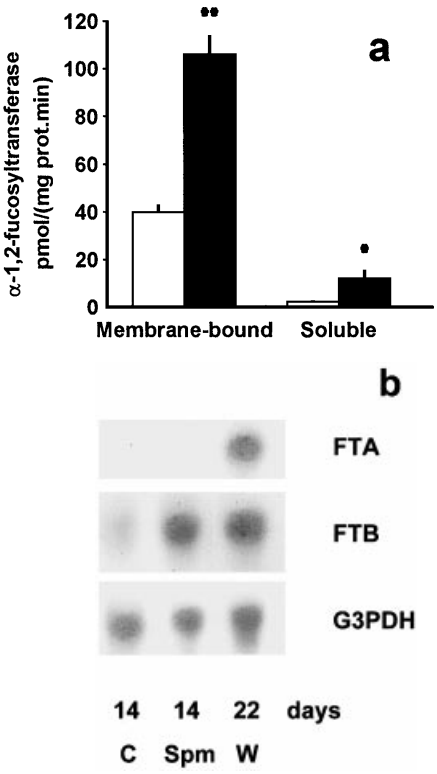


Figure 3 Effect of treatment of suckling rats with spermine on the activity and mRNA level of α-1,2-fucosyltransferase

Animals were given 0.4 μmol of spermine/g body weight orally once a day for 4 days starting on the 10th day of life (spermine group), or water (control group) in the same way. They were killed on the 14th day of life. (**a**) α-1,2-Fucosyltransferase activity (determined with phenyl-β-galactoside as acceptor) in microsomal membranes (membrane-bound) and in cytosol (soluble): black bars, spermine group; white bars, control group. Results are means $\pm$ S.E.M. ($n = 8$) and were compared by Student's *t* test. $^{*}P < 0.005$; $^{**}P < 0.002$. (**b**) Northern blots for the determination of α-1,2-fucosyltransferase mRNA. Hybridization was performed with the digoxigenin-labelled α-1,2-fucosyltransferases (FTA or FTB) cRNA probes; control hybridization was performed with digoxigenin-labelled G3PDH cRNA probe. Abbreviations: C, control 14-day-old suckling rats; Spm, spermine-treated 14-day-old suckling rats; W, 22-day-old weaned rats. Each lane was loaded with 25 μg of total RNA.

treated rats, in comparison with $30.4 \pm 2.9\,\%$ ($n = 10$, $P < 0.001$) for the control rats. In contrast, putrescine and spermidine levels were not significantly affected by the treatment. *N*-Acetylspermidine and *N*-acetylspermine levels in the intestine, which are normally low, were not significantly changed by the treatment in spite of an increasing trend. Results were similar when they were expressed as nmol/mg of protein.

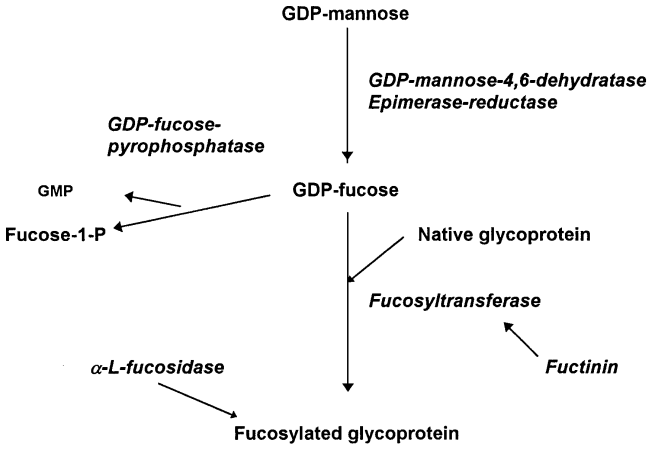
Effect of spermine treatment of suckling rats on sialyl- and fucosyl-transferase activities

When given intraperitoneally to suckling rats, spermine had no effect on glycosyltransferase activities (results not shown), whereas spermine treatment, via the oral route between the 10th and the 14th days of life, induced variations in at least one glycosyltransferase activity.

The specific sialyltransferase activity (determined with asialofetuin), in spite of a slight increasing trend, was not significantly modified by the treatment of suckling rats with spermine, as shown in Figure 2(a), whereas we observed a very large increase in fucosyltransferase activity (determined with asialofetuin as acceptor). The specific soluble and membrane-associated fucosyltransferase activities were increased by spermine (Figure 2b), reaching levels found in 22-day-old weaned rats [11]. When expressed in terms of total activity per g of intestine or per mg of mucosa DNA, the sialyltransferase and fucosyltransferase activities varied in an analogous way to that when expressed as specific activities (results not shown). This was because neither the protein content [41.8 ± 1.5 mg/g of intestine for the control group ($n = 6$) and 49.7 ± 7.2 mg/g of intestine for the spermine-treated group ($n = 6$)] nor the DNA content [5.7 ± 0.2 mg/g of intestine for the control group ($n = 6$) and 5.0 ± 0.3 mg/g of intestine for the spermine-treated group ($n = 6$)] was modified significantly by the oral treatment. These results suggest that the number of intestinal cells and their protein biosynthesis was not affected by spermine treatment in the conditions used in this study.

Effect of the treatment of suckling rats with spermine on α-1,2-fucosyltransferase activity and mRNA levels

The α-1,2-fucosyltransferase activity was determined more specifically by using *p*-nitrophenyl-β-D-galactoside as acceptor. The activity obtained with this specific acceptor was higher than that observed with asialofetuin. The α-1,2-fucosyltransferase



Scheme 1 Glycoprotein fucosylation process

Table 1 Effect of treatment with spermine on the enzymic and regulatory systems implicated in glycoprotein fucosylation

Animals were given 0.4 μmol of spermine/g of body weight orally once a day for 4 days starting on the 10th day of life (Spm group), or water (control group) in the same way. Results are means $\pm$ S.E.M. for six determinations; they were compared by the Mann–Whitney *U* test. $^*P < 0.005$.

Enzyme	Control group	Spm group
Fuctinin (units/mg of protein)	392 ± 29	326 ± 46
GDP-fucose synthesis (nmol/min per mg of protein)	31 ± 6	46 ± 13
GDP-fucose degradation (nmol/min per g of protein)	590 ± 98	850 ± 220
α-L-Fucosidase (pmol/min per mg of protein)	32.6 ± 2.6	$3.6 \pm 0.5^*$

activity was greatly increased by the oral spermine treatment; the membrane-bound and soluble forms of the enzyme were affected similarly (Figure 3a), at levels between those of 22-day-old weaned and adult rats [11].

Treatment with spermine induced a large increase in the intestinal content of mRNA for one of the α-1,2-fucosyltransferases, as shown after Northern blotting (Figure 3b). Indeed, the level of mRNA coding for the α-1,2-fucosyltransferase FTB gene was increased by the treatment with spermine and attained a level near that of 22-day-old weaned rats and slightly lower than that of adult rats (results not shown): the ratio of FTB to G3PDH was 0.54 ± 0.27 for the 14-day-old control group, 1.27 ± 0.07 for the 14-day-old spermine-treated group and 1.52 ± 0.19 for the 22-day-old group (two or three independent determinations for each group). In contrast, the level of mRNA coding for the α-1,2-fucosyltransferase FTA gene (almost undetectable in suckling rats) was not affected by the treatment (Figure 3b).

Effect of spermine treatment of suckling rats on enzymic and regulatory systems involved in the fucosylation process

The fucosylation process involves several enzymic and regulatory systems leading to the biosynthesis of the intestinal fucoproteins, as shown in Scheme 1. The activity of fuctinin (a protein that inhibits the α-1,2-fucosyltransferase activity) was not modified by oral treatment with spermine (Table 1). However, the synthesis of GDP-fucose from GDP-mannose by epimerase–reductase and its degradation by GDP-fucose pyrophosphatase were not significantly affected by treatment with spermine, in spite of a slight increasing trend (Table 1). For these two enzymes, the rather large distribution of the results could be that several experimental steps were necessary (incubation, HPLC separation), thus increasing the error in comparison with the other enzymic determinations. Only the activity of α-L-fucosidase was strongly decreased, by a factor 9.1, after spermine treatment (Table 1).

Effect of spermine treatment of suckling rats on sialoproteins and fucoproteins in the brush border membrane

To study the effect of spermine on the different parameters involved in the sialylation and fucosylation processes, the presence of α-2,3- and α-2,6-sialoproteins and of α-1,2-fucoproteins was determined in the brush border membranes of 14-day-old rats treated orally with spermine (Spm group) or not (Control

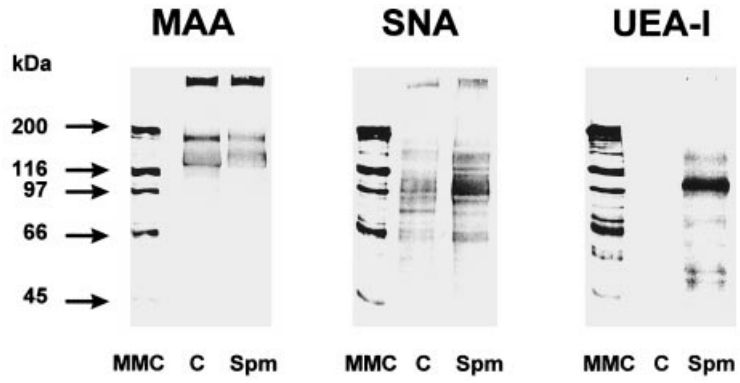


Figure 4 Effect of treatment of suckling rats with spermine on sialoproteins and fucoproteins in brush border membranes

Animals were given 0.4 μmol of spermine/g body weight orally once a day for 4 days starting on the 10th day of life, or water in the same way, then killed on the 14th day of life. Detection of sialoproteins and fucoproteins was performed after SDS/PAGE and transfer to nitrocellulose membranes of brush-border-membrane proteins obtained from 14-day-old control rats (C) or 14-day-old spermine-treated rats (Spm). MMC, molecular mass control (indicated at the left). For the detection of α-2,3-sialic acid residues with *Maackia amurensis* lectin (MAA), 15 μg of protein was loaded per lane; for the detection of α-2,6-sialic acid residues with *Sambucus nigra* lectin (SNA), 8 μg of protein was loaded per lane; for the detection of α-1,2-fucose residues with *Ulex europeus* lectin (UEA-I), 12 μg of protein was loaded per lane.

group). As shown in Figure 4, the α-2,3- and α-2,6-sialoproteins were similar in spermine-treated and control rats, although there was a slight decrease in the intensity of α-2,3-sialoprotein bands and a slight increase in those of α-2,6-sialoproteins in the spermine-treated group. In contrast, the α-1,2-fucoproteins were absent from the brush border membranes of the control 14-day-old pups but several α-1,2-fucoproteins (which were similar to those observed after weaning) appeared precociously in the brush border membranes of the 14-day-old rats treated with spermine, among which a protein with a molecular mass of 100 kDa was detected in particularly large amounts.

DISCUSSION

The rat small intestine is immature until the end of the third week after birth. This period corresponds to weaning, when suckling rats are fed on a solid diet containing a level of spermine higher than that of milk, as also described by Pollack et al. [34] for rat milk and by Romain et al. [35] for commercial food. We have shown that the intestinal level of spermine was increased markedly after weaning in 28-day-old weaned rats in comparison with 14-day-old suckling rats, as also observed by Luk et al. [15].

The aim of the present study was to assess the role of spermine as an intestinal maturation factor during postnatal development and its implication in the maturation processes of glycoprotein sialylation and fucosylation. Indeed, the increase in intestinal spermine level at weaning paralleled that of fucosyltransferase activity that is naturally observed at this period [8], and the appearance of fucoproteins in the brush border membranes [11].

In intestines of 14-day-old rats treated orally with spermine (at a concentration below the level of cytotoxicity and not exceeding the daily level eaten by weaned rats), the level of spermine was significantly higher than in the intestines of 14-day-old control rats and attained the level of weaned rats, as also described by Dufour et al. [36]. The levels of the other polyamines (spermidine and putrescine) and of *N*-acetylspermidine and *N*-acetylspermine were not modified. These results indicate that no important interconversion occurred from spermine to spermidine and to putrescine; they agree with those of Bardocz et al. [18], which showed that polyamines are readily taken up from the lumen and that spermine is not as strongly metabolized as putrescine. In suckling rats treated orally with spermine, the increase in the intestinal content of spermine in comparison with control rats is probably due to the intake, at the apical pole of the epithelial cells, of a higher quantity of this polyamine that is present in the gut lumen, rather than to a higher contribution via blood circulation, because intraperitoneal administration of spermine was without effect. Moreover, Dufour et al. [36] and Buts et al. [37] showed respectively either no modification or a decrease in the activity of the ornithine decarboxylase in suckling rats treated with spermine, indicating that the biosynthesis of spermine in enterocytes should not increase after the treatment of suckling rats with spermine.

We have studied the effect of treatment of suckling rats with spermine on the maturation of glycoprotein glycosylation. The sialyltransferase activity (determined on asialofetuin, an acceptor for both α-2,3- and α-2,6-sialic acids) was not significantly modified by the spermine treatment. The α-2,3-sialic acid content in glycoproteins of the brush border membranes seemed to be decreased very slightly by the treatment of 14-day-old rats with spermine, whereas the α-2,6-sialic acid content seemed sligtly increased. A study of the specificity of sialyltransferases could be interesting, although the major sialyltransferase described in young rats is an α-2,6-sialyltransferase [9]. In contrast, the soluble and membrane-bound fucosyltransferase activities (determined with asialofetuin, an acceptor for both α-1,2- and α-1,3-fucosyltransferases) were greatly increased after the oral administration of spermine; the α-1,2-fucosyltransferase activity (specifically determined with phenyl-β-D-galactoside) was increased similarly, reaching a level between those observed in weaned and in adult rats [11]. The mRNA level of one of the α-1,2-fucosyltransferases (FTB) known in rat was highly increased in 14-day-old spermine-treated rats in comparison with 14-day-old control rats and at a level near that observed for weaned rats. Given the stability of the fuctinin level in the spermine-treated rats compared with that in the control rats, the premature increase in α-1,2-fucosyltransferase activity is due to the transcriptional regulation of the expression of one of the α-1,2-fucosyltransferase rather than to the control of this activity by fuctinin. These changes, accompanied by the availability of a stable substrate and a large decrease in α-L-fucosidase, induced a parallel precocious appearance of several α-1,2-fucoproteins in the brush border membrane, similar to those observed after weaning [25]. It could be interesting to determine the nature of one of them (with a molecular mass of 100 kDa), which was more strongly revealed than the others and might be one of the glycoprotein enzymes of the brush border membrane (e.g. sucrase, maltase or aminopeptidase), also precociously induced after spermine ingestion [18,36]. However, it remains to be determined whether the precocious induction of intestinal fucosylation, observed in suckling rats treated orally with spermine, is due to a direct effect of polyamines after enterocyte penetration at the luminal side of the cell or to an indirect stimulated secretion of hormone or of other secondary effectors. Indeed, it has been suggested by some authors that spermine could affect the secretion of corticotropin ('ACTH') and corticosterone, probably by stimulating the release of gastrointestinal hormones [21], or it could be active through a cytokine-dependent mechanism [38].

The increase in intestinal glycoprotein fucosylation at weaning could be the result of the ingestion of a higher level of polyamines (particularly of spermine) given in a solid diet. However, the drastic change in diet composition at weaning might also induce modifications in the intestinal microflora. By comparing genetically identical germ-free mice with mice raised with a functional microbiota, Bry et al. [39] have shown that the production of fucosylated glycoconjugates, appearing in the small intestine and colon after the age of weaning, requires components of the microbiota. Fucoconjugates are largely absent from weaned germ-free mice; inoculation with flora restored the same fucosylation pattern as in conventional mice and induced an accumulation of α-1,2-fucosyltransferase mRNA. These results suggest that soluble mediators such as lipopolysaccharides and polyamines, synthesized by bacteria [40] and known to induce cell proliferation [16,41], might be involved in this process. Thus, in addition to an increased polyamine contribution due to the solid diet, polyamine changes due to modifications in the microflora might be involved in the increase in the fucosylation of glycoproteins at weaning. Moreover, the additional effect of variations in the biosynthesis of endogenous polyamines at weaning cannot be excluded, because ornithine decarboxylase is increased just after weaning [15]. The association of elevated levels of ornithine decarboxylase activity with increased cell proliferation is known [42], and a delayed intestinal maturation has been observed in the sparse-fur mouse (whose level of ornithine decarboxylase is low [43]), which is accompanied with lower levels of sucrase, trehalase and alkaline phosphatase. It is also not impossible that other hormonal factors (particularly insulin [11]) or maturation factors might have a role in the increase in fucosyltransferase activity at weaning, so that sperm-

ine might be one of several factors involved in such metabolic changes.

In conclusion, this study indicates that the sialylation and fucosylation of glycoproteins in small intestine are differently regulated, because the increased intestinal level of spermine, after oral ingestion of this polyamine by immature suckling rats, can induce a precocious and marked maturation of the glycoprotein fucosylation process (α-1,2-fucosyltransferase, α-L-fucosidase and α-1,2-fucoproteins) and only negligible changes in glycoprotein sialylation. Spermine is an important maturation factor for many glycoproteins in the brush border membrane, both in terms of enzyme activities and appearance (as described for sucrase and maltase) and in terms of fucosylation evolution, which reproduces the effect of weaning when given to suckling rats. Spermine, contained at a high level in solid food, could thus be important in postnatal intestinal glycoprotein fucosylation at weaning time and could be one of the hormonal and maturation factors involved in the drastic changes that occur during postnatal development.

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